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A Nonc valent Complex Vaccine Prepared with Detoxified *Escherichia coli* J5 (Rc Chemotype) Lipopolysaccharide and *Neisseria meningitidis* Group B Outer Membrane Pr tein Produces Pr tective Antib dies against Gram-Negative Bacteremia

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Earlier studies showed that purified IgG from sera of rabbits immunized with a boiled *Escherichia coli* J5 (Rc chemotype) whole cell vaccine protected neutropenic rats against gram-negative bacterial sepsis. In the present study, de-*O*-acylated J5 lipopolysaccharide (J5 DLPS) as a noncovalent complex with *Neisseria meningitidis* group B outer membrane protein (GBOMP) elicited anti-J5 LPS antibodies in rabbits. IgG prepared from immune rabbit sera protected neutropenic rats against lethal challenge with *Pseudomonas aeruginosa* 12:4:4 (Fisher Devlin immunotype 6). Sixteen of 26 rats treated with the postimmune serum IgG were protected compared with none of 20 rats treated with the control rabbit serum IgG ($P < .001$). In vitro binding studies showed binding of anti-J5 IgG to several gram-negative bacteria. These results indicate that a subunit vaccine made of J5 DLPS as a noncovalent complex with GBOMP may protect against gram-negative bacteremia.

There are ~400,000 cases of septicemia each year in the United States [1]. Gram-negative bacteremia occurs in ~30% of patients with septicemia [2]. Attempts have been made to develop vaccines that will protect against gram-negative bacteremia. Data from animal models suggested that immunizations with vaccines in which the core lipopolysaccharide (rough LPS) regions were exposed could protect against challenge with heterologous organisms [3–5]. Ziegler et al. [6] used the J5 mutant (Rc chemotype) of *Escherichia coli* O111:B4 to immunize human volunteers with the heat-killed bacteria. The administration of the immune human serum reduced deaths from gram-negative bacteremia in hospitalized patients (compared with pati nts receiving preimmune serum). Since this was a whole cell vaccine, the protective antigen was not clearly identified. In addition, whole cell vaccines have the potential for adverse reactions such as seen with typhoid and pertussis vaccines [7, 8].

We have previously shown that antisera from rabbits immunized with boiled *E. coli* J5 whole cell vaccine protect neutropenic rats against gram-negative bacteremia [9]. In a subsequent study [10], we showed that affinity-purified J5 LPS–specific IgG prepared from the serum of a rabbit immunized with boiled *E. coli* J5 whole cell vaccine protected neutropenic rats against challenge with *Pseudomonas aeruginosa* 12:4:4 (Fisher Devlin immunotype 6). The objectives of the present study were to determine whether a subunit vaccine prepared with de-*O*-acylated *E. coli* J5 LPS (J5 DLPS) as a noncovalent complex with *Neisseria meningitidis* group B outer membrane protein (GBOMP) would elicit high titers of anti-J5 LPS antibodies in rabbits and whether IgG prepared from such immune sera would protect neutropenic rats against lethal challenge with *P. aeruginosa* 12:4:4.

Materials and Methods

E. coli J5 LPS and lipid A from *E. coli* K12 were purchased from List Biological Laboratories (Campbell, CA). *P. aeruginosa* 12:4:4 was originally obtained from A. McManus (US Army Institute of Surgical Research, San Antonio, TX) and kept in the Walter Reed Army Institute of Research (WRAIR) collection. The LPS from this strain was prepared by the hot-phenol method of Westphal and Jann [11]. Phosphatase-labeled goat anti-rabbit IgG (heavy and light chains) and fluorescein isothiocyanate (FITC)–labeled goat anti-rabbit IgG were purchased from Kirkegaard & Perry Laboratories (Gaithersburg, MD). Protein G–Sepharose 4 fast flow (FF) was purchased from Pharmacia Biotechnology (Piscataway, NJ). Empigen BB was obtained from Albright & Wilson (Whitehaven, UK).

N. meningitidis group B 8529 was a case isolate from Chile and was maintained at −70°C in the WRAIR collection. Group B outer membrane protein (GBOMP) from this strain was prepared by a method described previously [12, 13]. Strains of *E. coli*, *Staphylo-*

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In conducting the research described herein, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" of the Institute of Laboratory Animal Resources, National Research Council.

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coccus aureus, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *P. aeruginosa*, *Enterobacter aerogenes*, and *N. meningitidis* 44/76, 8047, and 8566 were from the WRAIR collection. QS21 was obtained from Cambridge Biotech (Worcester, MA).

Preparation of J5 DLPS. J5 DLPS was prepared by a method described previously [14] with slight modification. Briefly, *E. coli* J5 LPS (10 mg) was dissolved in 0.1 *M* NaOH solution (4.5 mL) by sonication in an ultrasonic bath (model 5200; Branson, Danbury, CT) for 5 min. The slightly hazy solution was collected into a screw-capped tube and heated at 65°C ± 1° for 2 h. The cooled solution was neutralized with 1.0 *M* HCl to a pH of ~7.0. The released fatty acids were removed by dialysis against three changes of sterile water (500 mL each) over 2 days. The dialyzed J5 DLPS was lyophilized; the yield was 8.5 mg/10 mg of starting material. Fatty acid analysis of J5 LPS and J5 DLPS by gas-liquid chromatography showed that the ester-linked C-12 and C-14 fatty acids [15] were cleaved off by the process of de-*O*-acylation, as expected (results not shown).

Preparation of J5 DLPS-GBOMP noncovalent complex. *N. meningitidis* GBOMP solution (1.5 mL, 3.6 mg/mL) in TEEN buffer (0.05 *M* TRIS, 0.15 *M* NaCl, 0.001 *M* EDTA, 0.1% Empigen BB, pH 8.0) was added to a solution (4.0 mL, 0.8 mg/mL) of J5 DLPS in 0.9% NaCl. The mixture was kept at 5°C for 2 h and then dialyzed against 500 mL of sterile 0.9% NaCl without stirring for 2 days and then with stirring for 5 days at 5°C. The dialysis buffer was changed to 500 mL of fresh sterile 0.9% NaCl and dialysis continued for another 5 days. The dialysis buffer was changed once again to 500 mL of fresh sterile 0.9% NaCl, and dialysis continued for 20 h at 5°C.

The dialyzed solution (5.5 mL) was slightly hazy; it was filtered through a 0.45-μm membrane and stored at 5°C until use. The protein content of this complex was determined with BCA protein assay reagent [16]. The J5 DLPS content was determined by the phenol-sulfuric acid method [17] using J5 DLPS as the standard. The GBOMP-to-J5 DLPS ratio was 1:0.6 (wt/wt). For comparison, a noncovalent complex was prepared using native J5 LPS and GBOMP; the GBOMP-to-J5 LPS ratio of this complex was 1:0.4 (wt/wt).

Immunization of rabbits. New Zealand White rabbits (2-2.5 kg) were obtained from the Hazelton Research Products (Denver, PA). Blood was obtained from rabbits before immunization. Two rabbits were in each group. Group 1 rabbits were immunized with the J5 DLPS-GBOMP complex vaccine (25 μg of J5 DLPS) without QS21, the adjuvant; group 2 was immunized with the same dose of vaccine plus QS21 (50 μg). Group 3 rabbits received a lower dose (2 μg) of vaccine without QS21; group 4 received the vaccine (2 μg) plus QS21 (10 μg). Group 5 rabbits were immunized with 25 μg of J5 DLPS (without GBOMP) plus QS21 (50 μg); group 6 was immunized with GBOMP alone. All injections were intramuscular. Three doses of vaccine were given at 0, 2, and 4 weeks. Blood samples were obtained at time 0 (before immunization) and at 2 and 6 weeks after primary immunization.

ELISA. ELISAs were done in 96-well flat-bottom polystyrene microtiter plates (Costar, Cambridge, MA) by the method of Engvall and Perlmann [18] with slight modification. Briefly, wells were first coated with 50 μg/mL poly-L-lysine type VIIB (Sigma, St Louis) in PBS (0.01 *M* sodium phosphate, 0.14 *M* NaCl, 0.02% NaN_3), pH 7.4, at 37°C for 1 h. The wells were emptied and then overlaid with either J5 LPS or lipid A at 10 μg/mL in PBS for 3 h at 37°C. Excess binding sites were then blocked with 1% casein (Fisher Scientific, Columbia, MD) in PBS at 37°C for 1 h. The wells were washed with PBS between steps to remove unbound material.

The antigen-coated plates were incubated with serial 2-fold dilutions of primary antibodies for 16 h at room temperature (25°C). The plates were then incubated with the phosphatase-labeled secondary antibody for 20 h at room temperature. Disodium p-nitrophenyl phosphate (Sigma) at a concentration of 1 mg/mL in [illegible] *M* diethanolamine buffer, with 1 m*M* $MgCl_2$, pH 9.8) was used as substrate. Absorbance was read at 410 nm on a plate reader (Dynatech, Alexandria, VA). ELISA antibody titers were calculated by multiplying the reciprocal dilution of the serum [illegible] optical density (OD) at A_{410} of ~0.5, which is near the [illegible] the linear part of the OD dilution curve in our assay.

Rabbit pyrogenicity assay. This assay was done using New Zealand White rabbits (2-2.5 kg) by a standard procedure [illegible]

[illegible]m bactericidal [illegible] Bactericidal assays were [illegible] described [20, 21]. *N. meningitidis* group B [illegible]529 (B:15:P[illegible]), 44/76 (B:15:P1.16:L3,7,9), 8566 (B:4:P1.15:L3,7,9), and [illegible] (B:2b:P1.2:L2,4) were used as target bacteria. Bactericidal titers represented the reciprocal dilution of the serum showing [illegible] bacterial killing. The ability of anti-J5 IgG to mediate the [illegible] of *P. aeruginosa* 12:4:4 was assessed in a previously described opsonophagocytosis test [22]. Briefly, bacteria that were grown to mid-log phase were washed and added to wells that contained freshly isolated human neutrophils, normal human serum ([illegible]), and either anti-J5 or preimmune rabbit serum IgG in a total volume of 100 μL.

Samples were removed at time 0 and at 2 h and plated on trypticase soy agar (TSA) at 37°C overnight. For bactericidal assays, bacteria were added to different concentrations of normal human serum in the absence of neutrophils, and samples were taken for colony counts at time 0 and at 60 min.

Preparation of IgG. Protein G-Sepharose 4 FF (5 mL of wet gel) was washed on a sintered glass funnel with water (25 mL) and then with 25 mL of PBS. The washed gel was suspended in 15 mL of PBS and degassed under vacuum for 15 min. The degassed gel was packed in a small glass column; the bed volume of the packed gel was 4.5 mL. Immune rabbit serum (4 mL) was passed through the washed column for 3 cycles. The column was then washed with PBS until the A_{280} of the wash buffer was <0.01. IgG was then eluted from the column with 0.15 *M* glycine-HCl buffer, pH 2.52, until the A_{280} of the eluted fractions was <0.05.

The eluted fractions were immediately neutralized with [illegible] *M* TRIS to ~pH 7.0. The fractions with ODs > 0.1 were combined and concentrated by ultrafiltration on PM-10 membrane to a final volume of 8.0 mL. This solution was filtered through a 0.[illegible] membrane and stored at −20°C. IgG was also prepared in the same way from the preimmune rabbit serum.

Binding of IgG to heterologous gram-negative bacteria. Bacteria were grown overnight at 37°C on TSA plates. The following morning, bacteria were grown to log phase at 37°C in trypticase soy broth, washed with PBS, and adjusted to an OD of 0.3 at 550 nm, which corresponds to a concentration of ~10^8 cfu/mL. Bacteria were then mixed in 100-μL aliquots with an equal volume of either normal rabbit serum IgG or rabbit anti-J5 LPS serum IgG as previously described [23, 24]. Following incubation at 4°C for

30 min, bacteria were washed twice in PBS and mixed with FITC-labeled anti-rabbit IgG.

Bacteria were then incubated with the fluorescence-labeled antibody for 30 min at 4°C, washed in PBS, and resuspended in 1% (wt/v l) paraformaldehyde. Controls consisted of bacteria incubated with secondary antibody in the absence of either normal serum IgG or anti-J5 serum IgG. Aliquots of bacteria were treated with 10 μg/mL imipenem overnight at room temperature (prior to treatment with IgG) to expose core determinants.

The fluorescence of stained bacteria was quantified by analysis in a flow cytometer (FACScan II; Becton Dickinson, Sunnyvale, CA) as previously described [23]. At least 5.0×10^3 bacteria were analyzed in triplicate, and channels were assigned on a five-cycle log scale. Bacteria were evaluated by setting the gate such that nonspecific binding was <1% (mean channel fluorescence). Antibody binding was expressed as percentage of cells in the positive gate where nonstaining or negative cells were on the left.

Neutropenic rat model of sepsis. The neutropenic rat model has been described [9, 25]. Briefly, female Sprague-Dawley rats (125–175 g) were obtained from Charles River Breeding Laboratories (Wilmington, MA). Cefamandole (100 mg/kg) was given intramuscularly beginning 96 h before bacterial challenge on an every-other-day schedule. Cyclophosphamide (100 mg/kg) was given intraperitoneally at time 0 followed by a second dose of 50 mg/kg 72 h later. At time 0 and at 48 and 96 h, the challenge strain of *P. aeruginosa* 12:4:4 was given orally via an orogastric tube. All manipulations were done with animals under light CO_2 anesthesia. Rats were monitored for fever with a noncontact digital infrared thermometer (Horiba; Markson Science, Phoenix). All animals were bacteremic at the onset of fever.

The animals received antiserum or IgG at 9.0 mL/kg intravenously via tail vein at the onset of fever (temperature >38.0°C, usually day 5 or 6). Control animals received normal saline on the same schedule. The animals were observed daily for 12 days after the initial dose of cyclophosphamide, and deaths were recorded. All animals were bacteremic with *P. aeruginosa* 12:4:4 at the onset f fever as determined by blood culture.

Measurement of endotoxin content. Blood samples were collected, and serum endotoxin was measured as described previously [10].

Statistical methods. Statistical analyses of animal mortality following various treatments were done by Fisher's exact test. Serum endotoxin levels in the treatment groups were compared by one-way analysis of variance (Kruskal-Wallis). A two-sample *t* test was used to compare specific groups.

Results

Immune response in rabbits. Rabbits immunized with a 25-μg dose of J5 DLPS–GBOMP noncovalent complex vaccine without the adjuvant QS21 showed a higher fold-rise in ELISA antibody titers than did those immunized with the vaccine plus QS21 (table 1). However, in the lower-dose (2 μg) groups, there were no significant differences in ELISA antibody titers of sera from rabbits immunized with or without QS21. The 2 rabbits that were immunized with J5 DLPS plus QS21, but without GBOMP, showed only a 2-fold rise in anti-J5 LPS titer (table 1). None of the rabbits had any significant rise in anti-lipid A antibody titer (results not shown).

The rise in ELISA titer against the *N. meningitidis* GBOMP did not show significant differences between the high (25 μg) and low (2 μg) dose of vaccine with or without QS21 (table 2). Rabbits immunized with GBOMP alone (group 6) had a >800-fold rise in anti-GBOMP antibody (table 2) and no rise in anti-J5 LPS antibody (table 1).

Bactericidal activity. Bactericidal titers of pre- and postimmunization sera from rabbits immunized with J5 DLPS–GBOMP noncovalent complex vaccine against *N. meningitidis* group B strains showed that the maximum fold-rise in bactericidal activity was against the homologous group B meningococcal strain 8529 (from which GBOMP was prepared) and the closely related strain 44/76 (table 3). There was only a 2- to 4-fold rise or no rise in bactericidal antibody against the 2 heterologous *N. meningitidis* group B strains (8047 and 8566). In the experiment with *P. aeruginosa* 12:4:4, we observed a 50% reduction in the original inoculum with 20% anti-J5 IgG. With the addition of human neutrophils, there was a 98% reduction in of *P. aeruginosa* 12:4:4 colony counts. Addition of preimmune rabbit serum IgG resulted in 25% reduction in colony counts; however, addition of anti-J5 IgG resulted in a >1-log reduction in bacterial counts.

Protection of neutropenic rats. IgG prepared from the serum of rabbit 42374, immunized with the J5 DLPS–GBOMP noncovalent complex vaccine (without QS21), protected 10 of 18 rats compared with none of 8 rats treated with IgG prepared from the preimmunization serum of the same rabbit ($P < .001$, figure 1). IgG prepared from the postimmune serum of rabbit 44760, which was immunized with J5 DLPS–GBOMP complex plus QS21, protected 6 of 8 neutropenic rats compared with none of 12 rats treated with IgG prepared from the postimmune serum of rabbit 46277 (which showed no rise in anti-J5 LPS antibody). Thus, a total of 16 of 26 rats were protected by treatment with anti-J5 IgG, whereas none of 20 rats treated with control IgG (from both preimmune serum and from serum of a rabbit that showed no rise in anti-J5 antibody) survived. None of 11 rats treated with the anti-GBOMP IgG were protected (figure 1). The IgG concentration of samples infused were ~1.2 mg/mL in all the experiments. These results represent cumulative data from three experiments, and in each experiment the anti-J5 IgG showed significant protection of neutropenic rats.

Cross-reactivity of IgG antibodies. Purified IgG from the postimmune serum of rabbit 42374, immunized with J5 DLPS–GBOMP noncovalent complex vaccine, was used for studying the binding to heterologous gram-negative bacteria by fluorescence-activated cell sorting analysis. IgG prepared from preimmune rabbit serum was used as control. In the absence of treatment with antibiotic (imipenem) to expose the endotoxin core, the anti-J5 IgG showed enhanced binding to at least 7 of the bacterial strains, including *P. aeruginosa* 12:4:4, the challenge strain used in the neutropenic rat model f sepsis

Table 1. IgG antibody titers to *E. coli* J5 lipopolysaccharide (LPS) in sera from rabbits immunized with J5 de-*O*-acylated LPS (DLPS)–*N. meningitidis* group B outer membrane protein (GBOMP) noncovalent complex vaccine.

Group no., vaccine	Rabbit no.	Titers Pre	Post	Fold-rise
1. DLPS (25 μg) + GBOMP without QS21	44660	96	3430	[illegible]
	42374	52	[illegible]	[illegible]
2. DLPS (25 μg) + GBOMP + QS21 (50 μg)	44760	151	[illegible]	[illegible]
	44877	206	2688	[illegible]
3. DLPS (2 μg) + GBOMP without QS21	46170	32	1392	[illegible]
	46880	40	[illegible]	[illegible]
4. DLPS (2 μg) + GBOMP + QS21 (10 μg)	40004	33	[illegible]	[illegible]
	46298	305	[illegible]	[illegible]
5. DLPS (25 μg) without GBOMP + QS21 (50 μg)	46277	104	[illegible]	[illegible]
	46886	24	[illegible]	[illegible]
6. GBOMP only (50 μg)	806	32	[illegible]	[illegible]
	807	32	[illegible]	[illegible]

NOTE. Titers, determined by ELISA, are reciprocal dilution of serum with an OD of ~0.5 at A_{405} multiplied by absorbance value. Pre, before immunization; post, 2 weeks after 3rd vaccine dose.

(table 4); however, with antibiotic treatment, there was enhanced binding of anti-J5 IgG to all gram-negative bacteria tested. There was no enhanced binding to the gram-positive organism, *S. aureus,* which lacks endotoxin.

We chose to present the binding data as percentage of bacterial cells that bound anti-J5 or preimmunization antibody (table 4). There was little increase in percentage of *E. coli* J5 bacteria that bound anti-J5 antibody relative to preimmunization antibody. When the amount of antibody binding was examined by shift in mean channel fluorescence (MCF), however, there was a marked shift in fluorescence from an MCF of 36 for the preimmune serum IgG to 136 for the post–J5 immunization serum IgG in the imipenem-treated group. A similar shift in MCF was observed for all bacteria shown in table 4 except for *S. aureus* (as expected).

Serum endotoxin level. The mean endotoxin levels in sera of rats treated with anti-J5 IgG (3.78 ± 1.90 ng/mL) were significantly lower at 24 h than in rats treated with either anti-GBOMP IgG (13.41 ± 4.88 ng/mL, $P < .05$) or preimmunization serum IgG (20.66 ± 7.55 ng/mL, $P < .01$).

Rabbit pyrogenicity test. The test for pyrogenicity of the J5 DLPS–GBOMP noncovalent complex vaccine in rabbits showed that there was an average rise in temperature of 0.2°C by both 0.05 μg and 0.5 μg of J5 DLPS in the vaccine formulation. A 10-fold higher dose of 5.0 μg of J5 DLPS resulted in an average rise in temperature of 1.4°C. In contrast, 0.05 μg

Table 2. IgG antibody titers to *N. meningitidis* group B outer membrane protein (GBOMP) in sera from rabbits immunized with J5 de-*O*-acylated lipopolysaccharide–GBOMP noncovalent complex vaccine.

Group no., rabbit no.	Titers Pre	Post	Fold-rise
1. 44660	141	12,070	85
42374	79	26,137	330
2. 44760	434	25,472	58
44877	109	17,958	164
3. 46170	182	2588	14
46880	119	4294	36
4. 40004	116	13,145	113
46298	225	7577	33
5. 46277	72	119	[illegible]
46886	84	377	[illegible]
6. 806	15	13,145	876
807	25	20,569	822

NOTE. Vaccines are shown in table 1. Titers were determined by ELISA. Pre, before immunization; post, 2 weeks after 3rd vaccine dose.

Table 3. Fold-rise of bactericidal titers between pre- and postimmune sera from rabbits immunized with *E. coli* J5 de-*O*-acylated lipopolysaccharide–group B outer membrane protein noncovalent complex vaccine against different strains of *N. meningitidis* group B.

Group no., rabbit no.	Fold-rise in titers against strain: [illegible]529	[illegible]44/76	[illegible]047	[illegible]566
1. 44660	2	8	[illegible]	[illegible]
42374	16	4	[illegible]	[illegible]
2. 44760	32	32	[illegible]	[illegible]
44877	8	8	[illegible]	[illegible]
3. 46170	<2	2	[illegible]	[illegible]
46880	[illegible]	4	[illegible]	[illegible]
4. 40004	16	8	[illegible]	[illegible]
46298	8	8	[illegible]	[illegible]

NOTE. Vaccines are shown in table 1.

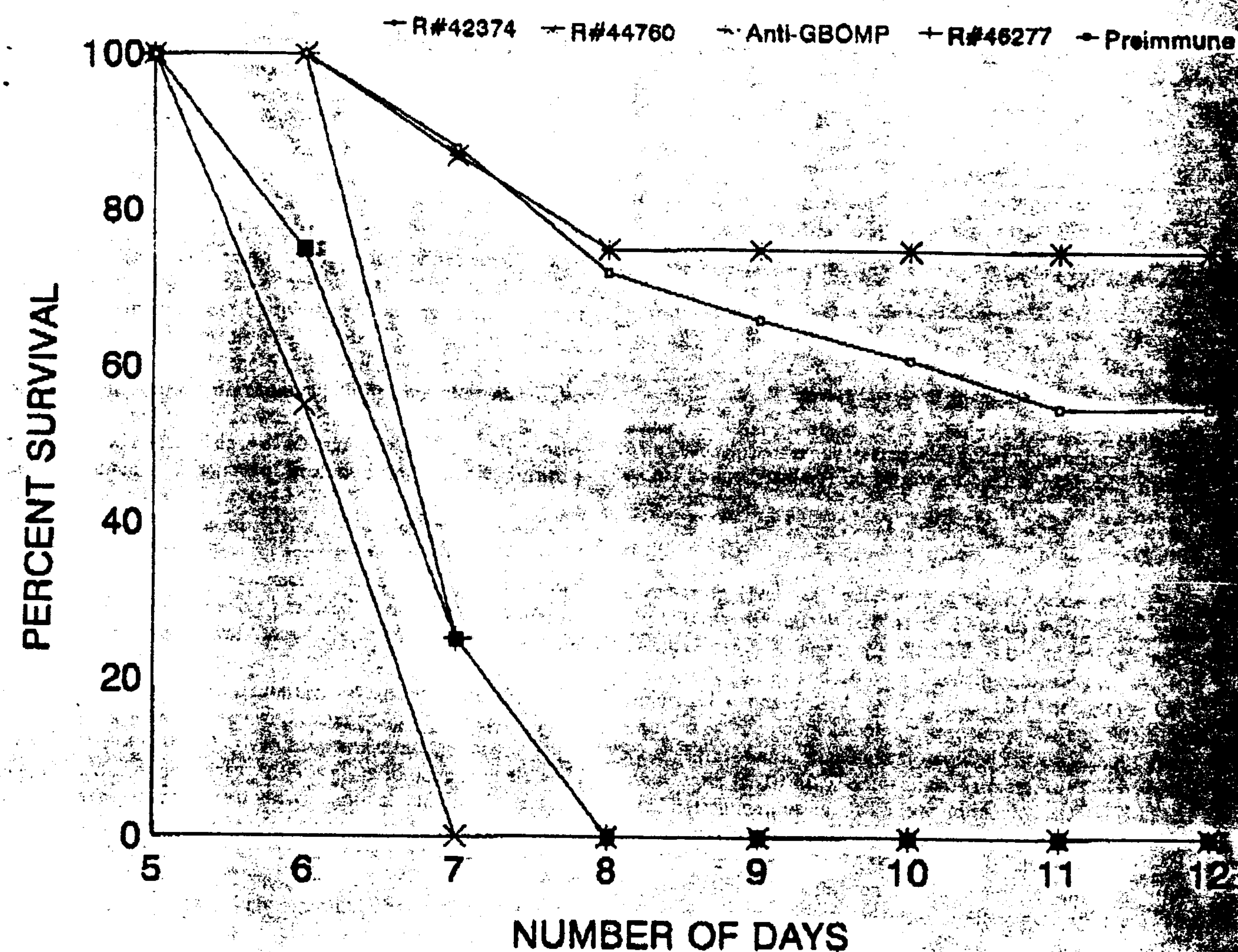

Figure 1. Protection of neutropenic rats with anti-J5 immunoglobulins against lethal challenge with *Pseudomonas aeruginosa* 12:4:4. All antibodies were given at 9 mL/kg. Rat of average weight of 150 g received 1.62 mg of IgG. Results are cumulative data from 3 experiments.

of a native J5 LPS–GBOMP noncovalent complex gave an average rise in temperature of 1.3°C. Thus, the DLPS complex was ~100-fold less pyrogenic.

Discussion

Gram-negative bacteremia is an important cause of mortality in hospitalized patients [26–28]. Extensive studies in animals [29–31] and limited studies in humans [6, 32] have shown that antibodies to the core determinants of gram-negative bacteria may protect against gram-negative bacteremia. We have previously shown that *E. coli* J5 LPS–specific IgG given as treatment protects neutropenic rats against gram-negative bacteremia [10]. The whole cell J5 vaccine used in these studies, however, is not suitable for routine use in humans. The subunit vaccine we used in the present studies consisted of purified *E. coli* J5 LPS that was detoxified by alkaline de-*O*-acylation (which removes ester-linked fatty acids) [33]. This treatment reduced the pyrogenicity of native J5 LPS ~100-fold.

Preliminary experiments using detoxified J5 LPS with alum as adjuvant showed it to be poorly immunogenic in rabbits, perhaps by covering up important epitopes (data not shown); consequently, we used GBOMP as adjuvant. The detoxified J5 LPS was formulated as a noncovalent complex with *N. meningitidis* GBOMP. The J5 DLPS–GBOMP noncovalent complex vaccine was highly immunogenic in rabbits. The anti-J5 LPS ELISA antibody titers of immune rabbit sera were comparable to the titers of sera from rabbits immunized with native J5 LPS–GBOMP noncovalent complex vaccine (data not shown). There was significant rise in anti-J5 LPS titer using this vaccine with and without the added adjuvant QS21 (table 1). In the absence of GBOMP, there was no significant enhancement of immunogenicity of J5 DLPS by the adjuvant QS21 [34]. The GBOMP has been shown to enhance immuno-

Table 4. Binding to imipenem-treated and -untreated whole bacteria of anti-*E. coli* IgG from pre- and postimmune sera of rabbit no. 42374.

Strain	Preimmune IgG		Postimmune IgG	
	No imipenem	Imipenem	No imipenem	Imipenem
E. coli				
J5	0.3	37.6	11.6	40.6
2961	22.3	20.1	35.9	57.8
2960	0.7	0	2.4	18.4
3037	0.4	0	1.1	22.9
2186	19.4	16.1	22.3	52.3
Staphylococcus aureus	10.6	6.7	6.8	1.4
Pseudomonas aeruginosa				
2967	0.4	3.4	1.0	35.0
12:4:4	7.2	39.5	44.3	81.1
134VA	12.5	22.5	80.1	91.5
2094	30.0	24.0	78.9	68.9
Enterobacter cloacae	0.4	2.2	0.9	55.0
Enterobacter aerogenes	5.3	3.4	11.8	41.7
Klebsiella pneumoniae 2085	23.3	37.4	65.9	95.3

NOTE. Data are % positive by fluorescence-activated cell sorting analysis.

genicity of peptide [35] and polysaccharide [36] vaccines when used as the noncovalent complex.

IgG prepared from the sera of rabbits immunized with J5 DLPS–GBOMP vaccine with or without QS21 showed significant protection of neutropenic rats (figure 1). That this protection was due to anti-J5 LPS antibodies was demonstrated by the fact that neutropenic rats were not protected by the passive transfer of high-titer anti-GBOMP IgG prepared from sera of rabbits immunized with GBOMP (figure 1). In addition, IgG prepared from serum of rabbit 46277, which did not have anti-J5 antibodies, failed to protect neutropenic rats. These results indicate that this subunit vaccine formulation generates protective anti-J5 antibodies in rabbits. Since the protection of neutropenic rats was against challenge with only 1 strain (*P. aeruginosa* 12:4:4) of gram-negative bacteria, it was necessary to determine the extent to which these anti-J5 antibodies bind to other potential gram-negative bacterial pathogens.

The binding assay using flow cytometry showed that anti-J5 IgG binds to clinical isolates of *E. coli*, *K. pneumoniae*, *Enterobacter* species, and *P. aeruginosa*, including the challenge strain 12:4:4, but not to a gram-positive coccus, *S. aureus* (table 4). IgG prepared from the preimmunization serum either did not bind or had significantly lower binding to the gram-negative bacteria compared with postimmune serum IgG. These results indicate that this vaccine may provide protection against other gram-negative bacteria such as *E. coli* and *Klebsiella* and *Enterobacter* species. Further work is in progress to test this hypothesis.

Our studies showed that a subunit vaccine consisting of a J5 DLPS–GBOMP complex induced antibodies that provided a level of protection similar to that previously observed with a killed whole cell J5 vaccine. The 100-fold reduction in pyrogenicity suggests that such a formulation may be well tolerated in humans. We are currently preparing this vaccine for human use, and a phase I clinical trial will be conducted as soon as preclinical animal experiments are completed.

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